lipids, **phosphatases** remove phosphate groups from nucleotides and phospholipids, and **nucleases** degrade DNA and RNA. Lysosomes are involved in the degradation of extracellular macromolecules that have been brought into the cell by **endocytosis** (Section E4), as well as in the degradation and recycling of normal aged cellular organelles, a process called **autophagy**.

### Peroxisomes

These organelles have a single boundary membrane and contain enzymes that degrade fatty acids and amino acids. A byproduct of these reactions is **hydrogen peroxide**, which is toxic to the cell. The presence of large amounts of the enzyme **catalase** in the peroxisomes rapidly converts the toxic hydrogen peroxide into harmless $H_2O$ and $O_2$:

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2$$

## Cytosol

The cytosol is that part of the **cytoplasm** not included within any of the subcellular organelles, and is a major site of cellular metabolism, containing a large number of different enzymes and other proteins. For example, glycolysis (Section J3), gluconeogenesis (Section J4), the pentose phosphate pathway (Section J5) and fatty acid synthesis (Section K3) all take place in the cytosol. The cytosol is not a homogeneous 'soup' but has within it the **cytoskeleton**. Also found within the cytosol of many cells are **inclusion bodies** (granules of material that are not membrane-bounded) such as glycogen granules in liver and muscle cells, and droplets of triacylglycerol in the fat cells of adipose tissue.

## Cytoskeleton

The cytoskeleton is an **internal scaffold** important in maintaining and altering the shape of the cell, in enabling cells such as sperm and white blood cells to move from one place to another, in transporting intracellular vesicles, and in pulling the chromosomes apart at mitosis and then dividing the cell in two. Three types of **filaments** make up the cytoskeleton: microfilaments, intermediate filaments and microtubules, each with distinct mechanical properties and dynamics.

### Microfilaments

The **microfilaments** (also known as **actin filaments**), diameter 5–9 nm, have a mechanically supportive function, determining the shape of the cell's surface and they are involved in whole cell movement. Microfilaments are two-stranded helical polymers of the protein **actin**, which appear as flexible structures organized into a variety of linear bundles and more extensive networks. Through their interaction with myosin, the microfilaments form contractile assemblies that are involved in various intracellular movements such as cytoplasmic streaming and the formation of membrane invaginations (Section B5).

### Intermediate filaments

The **intermediate filaments** (7–11 nm in diameter) provide mechanical strength and resistance to shear stress. They are made of **intermediate filament proteins**, which constitute a large and heterogeneous family, that form rope-like fibers. The skin in higher animals contains an extensive network of intermediate filaments made up of the protein **keratin** that has a two-stranded α-helical coiled-coil structure, while the nuclear lamina,

a meshwork just beneath the inner nuclear membrane, is formed from another type of intermediate filament.

### Microtubules

The third type of cytoskeletal filaments, the **microtubules**, determines the position of membrane-bound organelles and directs their intracellular transport. For example, the **mitotic spindle** involved in separating the replicated chromosomes during mitosis is an assembly of microtubules. Microtubules are hollow cylindrical structures with an outer diameter of 25 nm that are built from the protein **tubulin** (Figure 3). The rigid wall of a microtubule is made up of a helical array of alternating α- and β-tubulin subunits, each 50 kDa in size. A cross-section through a microtubule reveals that there are 13 tubulin subunits per turn of the filament. Microtubules in cells are formed by the addition of α- and β-tubulin molecules to pre-existing filaments or nucleation centers. One end of the microtubule is usually attached to a **microtubule-organizing center** called a **centrosome**. The drugs **colchicine** and **vinblastine** inhibit the polymerization of microtubules, thus blocking cell processes such as cell division that depend on functioning microtubules. Another compound, **taxol**, stabilizes tubulin in microtubules and promotes polymerization. Some of these compounds, such as vinblastine and taxol, are being used as **anticancer drugs**, since they block the proliferation of rapidly dividing cells by interfering with the mitotic spindle.

## Plant cell wall

Surrounding the plasma membrane of a plant cell is the cell wall, which imparts strength and rigidity to the cell. This is built primarily of **cellulose**, a rod-like **polysaccharide** of repeating glucose units linked β1–4 (Section J2). These cellulose molecules are aggregated together by hydrogen bonding into bundles of fibers, and the fibers in turn

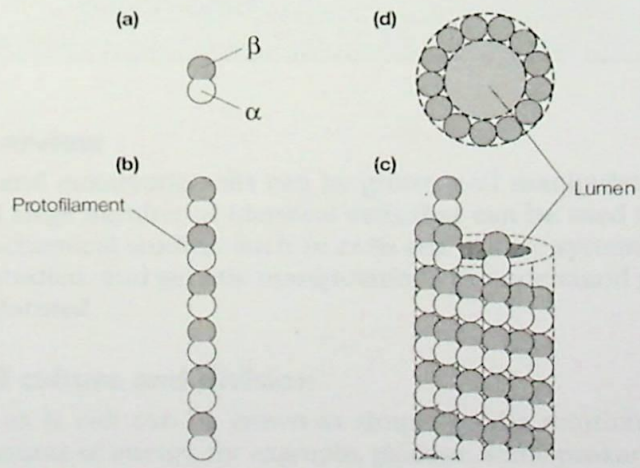


Figure 3. The structure of a microtubule: (a) tubulin consists of α- and β-subunits; (b) a tubulin protofilament consisting of many adjacent subunits; (c) the microtubule is formed from 13 protofilaments aligned in parallel; (d) cross-section of the hollow microtubule.

are cross-linked together by other polysaccharides. In wood another compound, **lignin**, imparts added strength and rigidity to the cell wall. Lignin is a complex water-insoluble phenolic polymer.

### Plant cell vacuole

Plant cells usually contain one or more **membrane-bounded vacuoles**. These are used to store nutrients (e.g. sucrose), water, ions and waste products (especially excess nitrogen-containing compounds). Like lysosomes in animal cells, vacuoles have an **acidic pH** maintained by $H^+$ pumps in the membrane and contain a variety of **degradative enzymes**. Entry of water into the vacuole causes it to expand, creating hydrostatic pressure (**turgor**) inside the cell, which is balanced by the mechanical resistance of the cell wall.

# A3 Cell growth



| Key Notes | |
|---|---|
| Cell culture: overview | Cells can be grown in culture under appropriate conditions, allowing cell biological studies and genetic manipulation to be undertaken. |
| Prokaryotic cell culture and division | Prokaryotic cells can be grown in simple media containing inorganic salts and a source of energy. In the prokaryotic cell cycle, the replication of DNA occurs as the cell enlarges, prior to division into two daughter cells by binary fission. |
| Eukaryotic cell culture | Animal and plant cells can be grown in culture but, in addition to inorganic salts and glucose, require various amino acids, vitamins and growth factors to survive. Primary cultures are prepared directly from tissues and have a finite life-span. Immortal cell lines derived from stem cells or tumor cells can proliferate indefinitely in culture. |
| Eukaryotic cell cycle | In eukaryotes, the cell cycle consists of M, $G_1$, S and $G_2$ phases. In the M phase, which lasts about 1 h, mitosis and cell division occur, while in the S phase the chromosomal DNA is replicated. Most of the cell cycle (95%) is spent in interphase ($G_1$, S and $G_2$ phases). Quiescent cells are said to be in the $G_0$ phase. |
| Related topics | (A1) Prokaryotic cells<br>(A2) Eukaryotic cells<br>(F2) Genes and chromosomes<br>(F3) DNA replication in bacteria<br>(F4) DNA replication in eukaryotes |

## Cell culture: overview

Both prokaryotic and eukaryotic cells can be grown and manipulated in **culture**. Cell culture provides a large number of identical cells that can be used for a variety of cell biological and biochemical studies. Such *in vitro* cell culture systems allow the growth of the cells to be studied, and genetic manipulations to understand gene structure and function to be performed.

## Prokaryotic cell culture and division

Prokaryotes such as *E. coli* can be grown in simple media solutions containing inorganic salts and a source of energy, for example, glucose. Some prokaryotes can replicate in approximately 20 min under ideal conditions (i.e. presence of an appropriate energy source, growth at the optimal temperature), which means that a single cell can multiply to many millions over a few hours.

The **prokaryote cell cycle** is relatively simple since the replication of the **DNA** in the **single circular chromosome** (Section A1) occurs continuously as the cell enlarges. The cell

then divides by **binary fission**. In this process, a new cell membrane and cell wall forms around the middle of the cell, which ultimately divides the parent cell into two daughter cells. Each of the daughter cells receives a copy of the chromosomal DNA.

## Eukaryotic cell culture

Although **yeast** can be grown readily in culture using minimal media as for bacterial cells, other eukaryotic cells (**animal and plant cells**) require much more complex culture media that contains in addition to salts and glucose, various amino acids and vitamins, which the cells cannot make themselves. Animal cell growth media also contains serum, which provides the various protein growth factors required to support cell division.

Animal cell cultures are initiated by the dispersion (for example by gentle homogenization, Section A5) of a piece of tissue into a suspension of the component cells. The isolated cells are then grown in a plastic **culture dish** under appropriate conditions with **defined growth medium**. Cultures prepared directly from the tissues of an organism are referred to as **primary cultures**. Such primary cultures can usually be cultured for 50–100 population doublings, after which they stop growing and die. In contrast, cells derived from tumors and embryonic stem cells frequently proliferate indefinitely in culture, and are referred to as permanent or immortal **cell lines**. Under the appropriate conditions, many cells in culture retain the differentiated properties appropriate to their origin. For example, fibroblasts continue to secrete collagen, and nerve cells extend axons. **Stem cells**, often cultured from early embryos, maintain their ability to differentiate into all of the cell types of the adult organism.

## Eukaryotic cell cycle

The division of eukaryotic cells must be carefully regulated with both cell growth and DNA replication being carefully coordinated in order to ensure the formation of daughter cells containing the complete complement of intact chromosomes (that is the complete genome, Section F2). The life of a eukaryotic cell can be defined as a **cell cycle**, which consists of four coordinated processes: cell growth, DNA replication, distribution of the duplicated chromosomes to daughter cells, and cell division.

**Mitosis** (nuclear division) in which the daughter chromosomes are separated and **cell division** (**cytokinesis**) occur in the **M phase**, which lasts for only about 1 h (Figure 1).

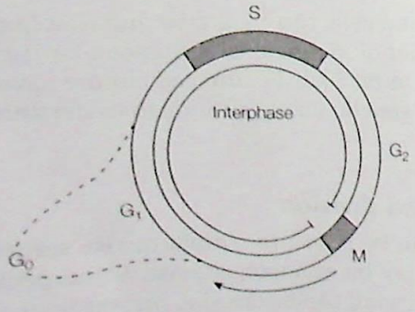


*Figure 1. The eukaryotic cell cycle.* The S phase is typically 6–8 h long, $G_2$ is a phase in which the cell prepares for mitosis and lasts for 2–6 h, mitosis (M) itself is short and takes only about 1 h. The length of $G_1$ is very variable and depends on the cell type. Cells can enter $G_0$, a quiescent phase, instead of continuing with the cell cycle.

The remainder of the cell cycle (approximately 95%) is spent in **interphase** (Figure 1), the period between mitoses. During interphase, the chromosomes are decondensed and distributed throughout the nucleus; at the molecular level, this is the time when both cell growth and DNA replication occur.

Mitosis is followed by the **$G_1$ phase** (G for gap) (Figure 1), which corresponds to the interval between mitosis and the initiation of DNA replication when the cell grows continuously. Then the cell enters the **S phase** (S for synthesis), during which time the chromosomal DNA is replicated, and finally the **$G_2$ phase** when cell growth continues and proteins are synthesized in preparation for mitosis. Eukaryotic cells in culture typically have cell cycle times of 16–24 h but the cell cycle time can be much longer (>100 days) for some cells in a multicellular organism. Most of the variation in cell cycle times occurs by differences in the length of the $G_1$ phase. Some cells *in vivo*, such as neurons, stop dividing completely and are said to be quiescent, locked in a **$G_0$ phase** (Figure 1).

# A4 Cell imaging

## Key Notes

**Light microscopy**

In light microscopy, a beam of light is focused using glass lenses to produce an enlarged image of the specimen. In bright-field microscopy, the specimen is illuminated from below with the beam of light being focused on to it by the condenser lens. The incident light that passes through the specimen is then focused by the objective lens on to its focal plane, creating a magnified image. The specimen to be viewed is first fixed with alcohol or formaldehyde, and then stained with a chemical, such as hematoxylin or eosin. Phase-contrast microscopy and the more complex differential interference contrast microscopy can be used to visualize living cells.

**Fluorescence microscopy**

In fluorescence microscopy, fluorescent compounds (which absorb light at the exciting wavelength and then emit it at the emission wavelength) are used to visualize a particular component in the cell. In confocal microscopy, a laser focuses light of the exciting wavelength on to the specimen so that only a thin section of it is illuminated. The laser beam is moved through the sample, producing a series of images, which are then reassembled by a computer to produce a three-dimensional picture of the specimen. Fluorescent compounds such as rhodamine and fluorescein can be coupled to antibodies that recognize the proteins of interest. The naturally occurring green fluorescent protein (GFP) from a jellyfish can be tagged on to other proteins and used to visualize the location and movement of proteins in living cells. Interactions between one protein and another can be monitored by fluorescence resonance energy transfer (FRET) by labeling the two proteins of interest with different fluorochromes. The lateral movement of a fluorescently tagged protein can be monitored by fluorescence recovery after photobleaching (FRAP).

**Electron microscopy**

In electron microscopy, a beam of electrons is focused using electromagnetic lenses. The specimen is mounted within a vacuum so that the electrons are not absorbed by atoms in the air. In transmission electron microscopy, the beam of electrons is passed through a thin section of the specimen that has been stained with heavy metals. The electron-dense metals scatter the incident electrons, thereby producing an image of the specimen. In scanning electron microscopy, the surface of a whole specimen is coated with a layer of heavy metal and then scanned with an electron beam to produce a three-dimensional image of the specimen.

| Related topics | (A2) Eukaryotic cells | (C4) Immunodetection |
|---|---|---|
| | (B6) Antibodies | (E2) Membrane structure |

## Light microscopy

In light microscopy, **glass lenses** are used to focus a beam of light on to the **specimen** under investigation. The light passing through the specimen is then focused by other lenses to produce a **magnified image**.

Several different types of light microscopy are routinely used to study various aspects of cell structure. The simplest is **bright-field microscopy** in which the specimen is illuminated from underneath by a lamp in the base of the microscope (Figure 1), with the light being focused on to the plane of the specimen by a **condenser lens**. Incident light coming through the specimen is picked up by the **objective lens** and focused on to its focal plane, creating a magnified image. This image is further magnified by the eyepiece, with the total magnification achieved being the sum of the magnifications of the individual lenses. In order to increase the resolution achieved by a compound microscope, the specimen is often overlaid with **immersion oil** into which the objective lens is placed. The limit of resolution of the light microscope using visible light is approximately 0.2 μm.

### Fixing and staining specimens

In bright-field microscopy the specimen to be examined is usually first fixed with a solution containing alcohol or formaldehyde. These compounds denature proteins and, in the case of formaldehyde, introduce covalent cross-links between amino groups on adjacent molecules, which stabilize protein–protein and protein–nucleic acid interactions. The **fixed specimen** may then be embedded in paraffin wax or a resin and cut into **thin sections** (0.5–10 μm thick) using a **microtome**. Each section is mounted on a glass slide and then positioned on the movable specimen stage of the microscope (Figure 1). The various subcellular constituents (nucleus, mitochondria, cytosol, etc.) absorb about the same degree of visible light, making it difficult to distinguish them under the light

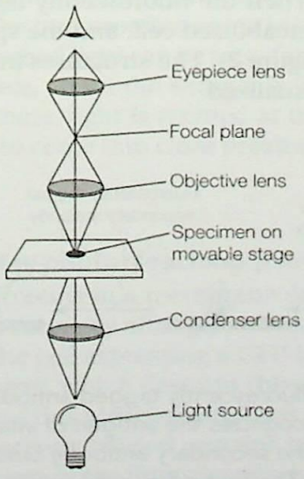


Figure 1. Optical pathway of a compound microscope.